

From Epidemiology, Clinical Medicine, Molecular Biology, and Atoms, to Politics: A Review of the Relationship between Thimerosal and Autism

(Submitted to the Institute of Medicine, of the US National Academy of Sciences, January 2004)

Authors:

David A. Geier, B.A.

President, MedCon, Inc.

and

Mark R. Geier, MD, Ph.D.

President, The Genetic Centers of America

Introduction

On July 1, 1999, the Food and Drug Administration (FDA) sent a letter to manufacturers of vaccines requesting their plans to remove thimerosal, a mercury-containing preservative present in vaccines since the 1930s, from US-licensed vaccines, or alternatively an explanation for continued use of thimerosal as a vaccine preservative. This was followed on July 7, 1999 by a joint statement issued by the American Academy of Pediatrics (AAP) and the US Public Health Service calling for the removal of thimerosal from vaccines. These actions were in part prompted by a risk assessment from the FDA concerning theoretical risk posed by potential cumulative doses of mercury children received from thimerosal-containing vaccines. The purpose of this review is to conduct a frank and impartial review of the scientific/medical evidence to determine, what, if any evidence exists concerning a relationship between thimerosal and the neurodevelopmental disorder of autism.

Autism

Autism is a lifelong neurological disorder that primarily strikes males. Communication and social interactions are severely impaired for persons with autism. Unable to learn from the natural environment as most children do, children with autism show little interest in the world or people around. Although some children with autism develop normal and even advanced skills, most exhibit a wide range of behavioral problems. Autism, in reality, is a lifelong developmental disability that profoundly affects the way a person comprehends, communicates, and relates to others.

As the first part of this review, we have examined the literature concerning the prevalence of autism in the US to determine if there has been any change in the prevalence of this disorder. Perhaps, one of the most comprehensive studies that has been undertaken on this subject was conducted by the California Department of Developmental Services. They have reported that since the 1980s, California has experienced dramatic increased in the number of children diagnosed with autism. Autism, once a rare disorder, was found to be more prevalent than childhood cancer, diabetes, and Downs' Syndrome. It was observed that the total number of persons with autism served statewide increased from 10,360 persons in December 1998 to 20,377 persons in December 2002. Between 1987 and December 2002, the population of persons with autism increased by 634 percent. In examining potential biases or confounders resulting in the increased prevalence of autism in the state of California, it was observed that population migrations, shifts in the interpretation of diagnostic criteria, or differences in diagnostic accuracy had limited effects on the increasing prevalence of autism. The

authors concluded that the increased prevalence of autism in California was a genuine phenomenon.

Independently, Blaxill et al. have analyzed the data generated from California to evaluate the changing prevalence of autism in that state. They report the hypothesis that “diagnostic substitution” can account for apparent increase in the incidence of autism in California is not supported by proper and detailed analyses of the California data. On the contrary, California, according to the authors, provides the strongest evidence for the explosion in the incidence of autism in the United States.

As a further confirmation of an increasing prevalence of autism, other studies outside of California have found similar increases in the prevalence rates of autism equal to or greater than those observed in California. Yeargin-Allsopp et al. reported that studies conducted in the US during the 1980s and early 1990s found that the prevalence of autism to be approximately 4 per 10,000 children. Yeargin-Allsopp et al. studied the prevalence of autism among children aged 3 to 10 years in Atlanta, Georgia in 1996. They determined that the prevalence of autism was 3.4 per 1,000 children, and that there was a higher prevalence of autism among the children aged 5-8 years-old in comparison to children 9-10 years-old. The authors concluded that the rate of autism found in their study was higher than the rates from studies conducted in the US during the 1980s and early 1990s, and was more consistent with recent studies. Bertrand et al. have evaluated the prevalence of autism for a defined community, Brick Township, New Jersey, using current diagnostic and epidemiologic methods. The authors evaluated a target population of children who were 3 to 10 years of age in 1998, who were residents of Brick Township at any point during that year. It was determined that the prevalence for children whose condition met full diagnostic criteria for autistic disorder was one in 250 children, and there was a higher prevalence rate of autistic disorders among children 3-5 years (1 in 181 children) in comparison to children 6-10 years (1 in 323 children).

We have previously analyzed the US Department of Education statistics and showed that there was an increase of 714 percent in the total number of autistic children in the US Department of Education from 1992-1993 to 2001-2002. We have also analyzed a single report of the US Department of Education, so as to minimize potential year-to-year differences in diagnostic criteria, and found a considerably higher prevalence of autism in younger children in comparison to the older children.

Yazbak has reviewed trends in autism in recent years. He has concluded that autism, once a rare disorder, has reached epidemic proportions in the United States. The increase cannot be attributed to changes in diagnostic criteria, which have actually become more restrictive, or as the result of a genetic epidemic because there are no genetic epidemics. Yazbak reported that the present autism epidemic appears to be the result of environmental etiological factors such as thimerosal in childhood vaccines. This conclusion is supported according to Yazbak because parents in increasing numbers have reported similar stories regarding the development of autism in their children. Namely, a child, most often a boy, who is developmentally, socially, and verbally on par for his age, suddenly stops acquiring new words and skills in the second year of life and then regresses, losing speech, cognitive abilities, and social dexterity.

Therefore, it is apparent that in recent years a very large and real increase in the number of children with autism has occurred, especially among younger children, and potential biases or confounders have had a minimal impact on the observed increase. It is

clear the epidemic cannot be a genetic epidemic, because genetics in a given population does not change that rapidly. In order to account for the increase, it must be due to an environmental factor that underwent a rapid evolution during the 1990s. The cost of the epidemic has already been estimated to be between two and 20 trillion dollars by such individuals as Congressman Dan Burton.

Biological Plausibility

Theoretical

In 2001, Bernard et al. put forth a medical hypothesis that autism was a novel form of mercury poisoning. The authors reported that exposure to mercury can cause immune, sensory, neurological, motor, and behavior dysfunctions similar to traits defining or associated with autism, and the similarities extended to neuroanatomy, neurotransmitters, and biochemistry. It was determined that thimerosal-containing vaccines have become a major source of mercury in children who, within their first two years, may have received a quantity of mercury that exceeds safety guidelines. In a different publication, Bernard et al. reported that the discovery and increase in the reported prevalence of autism parallels the introduction and spread of thimerosal-containing vaccines. Autism was first described among children born in the 1930s. Thimerosal was first added to childhood vaccines in the 1930s. Prior to 1970, autism was estimated to occur in approximately one in 2,000 children, while the average prevalence of autism increased to about one in 1,000 children from 1970 to 1990. This was a time period of rapid increased immunization coverage in the US. By 1995, the National Institute of Health reported that the prevalence of autism to be one in 500 children, and in 2000 it was determined that the prevalence of autism was one in 250 children. The authors reported in the early 1990s that thimerosal-containing *Haemophilus influenzae* type B (Hib) and pediatric hepatitis B vaccines became part of the routine infant schedule. In addition, the authors reported that the onset of autistic symptoms generally follows the administration of thimerosal in vaccines, and symptom emergence is consistent with expression of mercury toxicity. Mercury exposure from vaccines began at birth and continued approximately at 2, 4, 6 and 15 months. The great majority of autistic children appear normal at birth, but subtle abnormalities in movement have been observed as early as four months of age, and sensory-motor disturbances are detected at 9-12 months. The full array of diagnostic impairments is generally evident by 15-24 months. Symptoms of mercury toxicity can arise suddenly in especially sensitive or sensitized individuals, but expression is usually gradual. Similarly, autistic symptoms usually emerge gradually, although there are instances of sudden onset.

The National Toxicology Program, of the National Institutes of Health, has reported that symptoms of thimerosal exposure include coordination disorders, behavioral changes, speech/language disorders, and mental retardation in children. The National Toxicology Program has also reported that thimerosal is a poison by ingestion, subcutaneous, intravenous, and possible other routes. Thimerosal has been shown to be an experimental neoplastigen, teratogen, and to have experimental reproductive effects. The eighth edition of *The Merck Index* states regarding ethylmercuric chloride, "Human Toxicity: All alkyl mercurials of this general type are highly toxic...Chronic exposure has caused permanent injury to brain."

Redwood et al. have reported that mercury is considered to be one of the most toxic metals in the world. Neurobehavioral alterations, especially to the more susceptible

fetus and infant, are known to occur after relatively low dose exposure to organic mercury compounds. In effort, to further elucidate the levels of ethylmercury resulting from exposure to thimerosal-containing childhood vaccines, the authors estimated hair mercury concentrations expected to result from the recommended US childhood immunization schedule utilizing a one compartment pharmacokinetic model. This model was developed to predict hair concentrations from acute exposure to methylmercury in fish. The authors determined that modeled mercury hair concentrations in infants exposed to thimerosal-containing childhood vaccines were in excess of the Environmental Protection Agency (EPA) safety guidelines of 1 part-per-million (ppm) for up to 365 days, with several peak concentrations within this period. More sensitive individuals and those with additional sources of exposure would have higher mercury concentrations. In conclusion, the authors stated given that exposure to low levels of mercury during critical stages of development has been associated with neurodevelopmental disorders, the predicted hair mercury concentration resulting from thimerosal-containing childhood vaccines is cause for concern.

Ball et al. from the Food and Drug Administration (FDA) have previously evaluated doses children received from thimerosal-containing childhood vaccines administered during the first six months of life. The authors determined that some children were in excess of some of the federal safety guidelines established for the oral ingestion of methylmercury. We have previously evaluated the instantaneous excess doses of mercury children received from thimerosal-containing childhood vaccines in comparison to the EPA (0.1 micrograms of methylmercury orally ingested/Kg bodyweight/day) and FDA (0.4 micrograms of methylmercury orally ingested/Kg bodyweight/day). We showed that children received in some cases more than 100-fold instantaneously in excess of some of the federal safety guidelines established for the oral ingestion of methylmercury. It was observed children received instantaneous doses of mercury from thimerosal-containing childhood vaccines throughout the childhood recommended immunization schedule (i.e. birth to 5 years-old) in considerable excess of both the EPA and FDA established safety guidelines for the oral ingestion of methylmercury.

Rohyans et al. have reported that the organic mercury present in merthiolate is easily absorbable, and undergoes widespread tissue distribution. Toxicity may be related both to the biotransformation into inorganic mercury and to the unchanged compound, both of which cause degenerative changes in the brain, especially in the visual cortex and cerebellum, and proliferative changes throughout the cerebellar context. The authors stated that there are definite individual differences in sensitivity to the effects of mercurials. Some patients tolerate prolonged exposure without symptoms; others have significant systemic signs and neurologic disability with much less exposure. Rohyans et al. also reported that although aqueous merthiolate has been used for years as a topical antiseptic, a recent review of its use by the FDA has resulted in its classification as "less than effective." Furthermore, thimerosal present in merthiolate is toxic if absorbed or injected.

Stetler et al. from the Centers for Disease Control and Prevention (CDC) have previously evaluated higher concentrations of mercury than those present in a single dose of whole-cell Diphtheria-Tetanus-Pertussis (DTP) vaccine (i.e. 25 micrograms of mercury per dose). They concluded that they had serious reservations about administering

higher doses of mercury from thimerosal-containing childhood vaccines because of, “the need to assure safety of the preservative.”

Methyl- and Ethylmercury

It has been previously reported by a number of authors that methylmercury and ethylmercury possess similar properties. Tan and Parkin have reported that ethylmercury ions and methylmercury ions should display similar complexation and chemical characteristics. The *Handbook on the Toxicology of Metals* states the available data indicate that ethylmercury compounds have toxicological properties similar to those of methylmercury compounds. Fagan et al. have published that although thimerosal is an ethylmercury compound, it has similar toxicological properties to methylmercury and the long-term neurological sequelae produced by the ingestion of either methyl- or ethylmercury based fungicides are indistinguishable. Zhang has reported that ethylmercury compounds have toxicological properties similar to those of methylmercury compounds, and there is evidence that both methyl- and ethylmercury can persist in the body for a long time. Yonaha et al. have reported that the clinical signs and pathological findings caused by methylmercury compounds in animal experiments are known to be similar to Minamata disease manifested in humans. At the same time, the symptoms in cats, calves, and mice poisoned by ethylmercury compounds are similar to those in methylmercury compounds. Further, alkylmercury compounds having short carbon chains (C₁-C₃) bring about specific neurotoxicity and signs of poisoning in rats including weight loss, ataxia, and closing of the hind legs. The INERIS has reviewed the effects of mercury and its derivatives. The report concluded that epidemiological studies carried out after accidents took place in Japan, Iraq, Canada, and New Zealand showed that methyl- and ethylmercury damaged the brains of babies exposed *in utero*. The most severe malformations (paralysis, delayed growth, and blindness) were observed in babies exposed during the second three-month period of pregnancy. Research on monkeys and rats showed that organic mercury was teratogenic at doses not toxic to the mother. The effects were hydrocephaly, facial deformation, and delayed ossification. Prenatal exposure can be associated with behavior problems, but also with functional changes to the kidneys, liver, and immune system. Even authors from the FDA have published, “Because higher-dose exposure to ethylmercury from thimerosal results in toxicity comparable to that observed after high-dose exposure to methylmercury, and because of the chemical similarity of the 2 compounds, it appears reasonable to consider toxicity of low doses of methylmercury and ethylmercury to be similar.”

Magos et al. have reported that the neurological signs and symptoms of methyl- and ethylmercury intoxication are identical and both produced similar neurotoxicities following *in vivo* administration in mice. Similarly, Ueha-Ishibashi et al. have conducted studies with thimerosal and methylmercury demonstrating that both had similar *in vitro* toxic effects on cerebellar granule neurons dissociated from 2-week-old rats.

An international committee has previously evaluated the maximum allowable concentrations of mercury compounds. The authors reported that the elimination of methyl- and ethylmercury is very slow, especially in man and primates, and consequently there is a considerable risk of mercury accumulation. It was determined that women of childbearing age should not be exposed to an occupational risk from methyl- and ethylmercury compounds. The authors concluded that for methyl- and ethylmercury salts, the ceiling value for mercury in whole blood should not exceed 10 micrograms of

mercury/100 mL, as total mercury. It should be noted, as an example, that some 2 month-old children received 62.5 micrograms (25 micrograms from DTP and Hib vaccines, and 12.5 micrograms from a hepatitis B vaccine) of mercury from the recommended childhood immunization schedule. Using Geigy Scientific Tables, it may be determined that there were 433.3 mL of whole blood present in the children (determined because a 3 month child has 87 ml/Kg of whole-blood, and the average child weighs 4.98 Kg). Applying the occupational ceiling of ethylmercury derived from the international conference, children received $([62.5 \text{ micrograms of mercury from the vaccines} / (433.3 \times 0.1)])$ 1.4-fold in excess of the allowable limit assuming that the mercury uniformly distributes in the body. Similarly, using Geigy Scientific Tables, it may be determined that there were 349 mL of whole blood present in the children (determined because in normal men the blood volume as a percentage of body weight is 7.0, and the average 2 month children weighed 4.98 Kg). Applying the occupational ceiling of ethylmercury derived from the international conference, children received $([62.5 \text{ micrograms of mercury from the vaccines} / (349 \times 0.1)])$ 1.8-fold in excess of the allowable limit assuming that the mercury uniformly distributes in the body. It must be kept in mind that an occupational exposure is far in excess of what should be allowed in a developing infant, which has been shown to be far more sensitive to mercury than adults. Additionally, this occupational exposure limit is one that was established in the 1960s, and since that time the allowable doses of mercury exposure have steadily decreased.

Miller et al. have investigated the distribution and excretion of methyl- and ethylmercury in animal systems. The authors intramuscularly injected chicks with 3.0 mg of methyl- and ethylmercury per kilogram of body weight. It was determined that higher concentrations of mercury were observed in the liver, blood, and kidney of chicks following ethylmercury injection than methylmercury injection. Similar decreased blood mercury concentrations were observed following injection of chicks with methyl- or ethylmercury, and significantly higher concentrations of mercury were present in the kidney and liver of ethylmercury injected chicks in comparison to methylmercury injected chicks 1-10 days following injection.

Brooks et al. developed a precise and accurate method for the determination of either methyl- or ethylmercury in the blood and tissue of rats using capillary gas chromatography with electron-capture detection. The authors applied their method to evaluate the pharmacokinetic study in rats dosed orally with 8 mg mercury/kg as methylmercury chloride and ethylmercury chloride. The authors found that higher concentrations of mercury present in the blood of ethylmercury (~100% of the dose entered the blood) treated rats than methylmercury (~80% of the dose entered the blood) treated rats. The authors also determined that the peak mercury blood concentration occurred sooner in methylmercury treated rats (12 hours) in comparison to ethylmercury (24 hours), and that greater amounts of mercury were present in the blood for longer times in ethylmercury (at 5 days: ~75% of maximum value) treated rats in comparison to methylmercury (at 5 days: ~60% of maximum value) treated rats.

Syversen has evaluated the distribution of mercury in the developing rat brain after injections of methylmercuric chloride and diethylmercury (the author stated regarding the diethylmercury that in light of the fact that in the present experiment the diethylmercury dose was given intraperitoneally, it is likely that the diethylmercury was transformed to ethylmercury before it reached the blood). Wistar rats were given

radiolabeled methylmercuric chloride and diethylmercury in peanut oil intraperitoneally (5.0 mg / Kg) every second day from 5 until 27 days of age. A reference group was given injections of oil only. It was observed that the methylmercuric chloride and diethylmercury groups had an equal mercury content in the brain, whereas no mercury was present in the examined brains of the reference group.

Demonstrated

Warkany and Hubbard have reported, "In several children of our series and in some recently reported, various immunization procedures preceded the onset of acrodynia in addition to mercurial exposure. This could be purely coincidental or the vaccination may play a role as an accessory factor. It is noteworthy that many vaccines and sera contain small amounts of mercury as preservatives which are injected with the biological material."

Derban et al. investigated 144 cases (age range: 1 ½ years- 74 years) of alkylmercury poisoning in a rural Ghana village of 250 persons. The patients out of ignorance had ingested maize that was dressed with ethylmercuric chloride. They all showed the usual clinical features of alkylmercury poisoning, and 20 persons died. Four children developed disturbances of speech that lead to stammering and scanning. Mental abnormality was observed in one boy who showed occasional outbursts of anger unrelated to circumstances.

Zhang has reported on clinical observations in ethylmercury chloride poisoning. Forty-one patients in the Peoples Republic of China were poisoned by ethylmercury chloride, caused by ingestion of rice that had been treated with the chemical. The onset of symptoms occurred on about day 7 after ingestion in some patients, but for most, symptoms started about day 15 (latent period range: 7-30 days). A wide-range of symptoms was observed in patients following ethylmercury chloride exposure, including significant neurological symptoms, with the most severe symptoms resulting in patient death. The author was able to generate a dose-response effect between increasing doses of ethylmercury chloride and increasing severe neurological symptoms.

Cinca et al. have reported on four cases of patients who ate the meat of a hog inadvertently fed seed treated with fungicides containing ethylmercury chloride. The clinical, electrophysiological, and toxicological, and in two of the patients the pathological data, showed that this organic mercury has very high toxicity not only for the brain, but also for the spinal motoneurons, peripheral nerves, skeletal muscles, and myocardium. Pfab et al. have reported on a 44-year-old man who ingested 83 mg/kg thimerosal. He developed gastritis, renal tubular failure, dermatitis, gingivitis, delirium, coma, poly neuropathy, and respiratory failure. Treatment was symptomatic plus gastric lavage and oral chelating agents were employed. The patient completely recovered. Maximum mercury concentrations were blood 14 mg/L, serum 1.7 mg/L, urine 10.7 mg/L and cerebrospinal fluid 0.025 mg/L. Mercury concentration in blood declined with two velocities: first with half-time 2.2 days, then with half-time 40.5 days. Lowell et al. have described a case of mercury poisoning associated with thimerosal-containing hepatitis B immunoglobulin (HBIG). A 44-year-old man with decompensated cirrhosis from hepatitis B underwent liver transplantation. He received 50 mL HBIG during the operation; 50 mL post operatively, and on days 2 and 3. On day 4 he was put on 10 mL HBIG intravenously, daily. On day 3 he complained of paranoid thoughts and had difficulty speaking. Neurological examination was normal. Over the next two days, his

speech became slow and slurred, although he had full comprehension. The only neurological finding was slow movements. A magnetic resonance image of the brain was normal. The electroencephalogram revealed generalized slowing. Nine days after transplant he developed resting tremor in both hands, progressing to choreiform movements of his upper extremities. He continued to make no attempts to speak or make any sounds, although he remained fully alert. He was eventually started on chelation therapy. Four weeks after initiation of chelation therapy he began to make sounds, and after five weeks he was able to walk without assistance, and speak clearly. The authors reported that HBIG contains thimerosal (a mercury-containing compound), and previous authors had demonstrated that administration of thimerosal-containing products might lead to mercury poisoning. Axton has reported on six cases of poisoning after administration of merthiolate. It was observed that five out of the six patients died. The author determined that the symptoms and clinical course of the six patients was suggestive of sub-acute mercury poisoning. In acute poisoning, death is usually rapid, as a result of cellular enzyme poisoning by the mercuric ion. In chronic poisoning, where small amounts of mercury ingested over a long period symptoms are mainly neurological. In intermediate forms of poisoning the kidneys are usually affected, as in the cases reported by the author, but in none of these cases could the renal damage have been the immediate cause of death according to the author.

Kiffe et al. have characterized the cytotoxic and genotoxic effects of thimerosal in CHO K5 cells with the comet assay (single-cell gel electrophoresis assay). Thimerosal was found to be positive in the standard and/or the all cell comet assay. The authors concluded overall that thimerosal was judged positive in the comet assay. Takahashi evaluated the cytotoxicity of thimerosal using Chang's human conjunctival epithelia in cell culture. The cultured cells were exposed for five seconds, two minutes, and 24 hours to thimerosal at various concentrations, obtained by serial dilutions. The cytopathic effect and cell desquamation from the wall of the culture flasks were observed with an inverted microscope and LD₅₀ was calculated by Van der Waerden's method. The experiments showed thimerosal was cytotoxic, inducing the following LD₅₀ values of 291.6, 47.4, and 2.2 :g/mL at exposure times of five seconds, two minutes, and 24 hours.

Mukai undertook an autoradiographic study in order to evaluate the distribution of ethylmercuri-S-cysteine (EMC) cells of the central nervous system. Mice were injected intraperitoneally with EMC labeled with tritium at a concentration of 0.3 mg/0.5 mL saline per day. The extent and distribution of cell damage were highly predictable, and selective necrosis of the small granular neurons in the koniocortex and neostriatum was a constant finding. Autoradiographic study suggested that the astroglial cell compartment played a role in conveying the mercury-protein complex into neurons. Uchida et al. evaluated the effects of injection of thimerosal solution on non-sensitized animals. Intrafootpad injection of thimerosal solution in non-sensitized mice resulted in a swelling response, which peaked 1 hour after injection and lasted for more than 24 hours. Histopathological examination showed that there were severe edema and infiltration of polymorphonuclear neutrophils at the site of injection. An increased vascular permeability was observed after cutaneous injection of thimerosal solution on the back of non-sensitized rats. Since mercuric chloride and methylmercury induced severer reactions, and thiosalicylic acid had no effect, mercury contained in thimerosal would have caused the reactions observed. The authors concluded that their results suggest that part of the

hypersensitivity reactions against thimerosal observed among patients were possibly induced by the toxic effect of thimerosal, and thimerosal contained as a preservative in vaccines may augment the side-effects of immunization. Nelson and Gottshall examined pertussis vaccines preserved with 0.01% merthiolate administered to mice. The authors observed that pertussis vaccines preserved with 0.01% merthiolate were more toxic for mice than unpreserved vaccines prepared from the same parent concentrate and containing the same number of organisms. An increase in mortality was observed when merthiolate was injected separately, before or after an unpreserved saline suspension of pertussis vaccine.

Animal Model of Thimerosal Exposure Resulting in Autism

Chian and Lipkin have investigated the possibility that thimerosal in vaccines results in aberrant brain development and behavior features reminiscent of autism in immature, susceptible hosts. The authors found that early postnatal administration of thimerosal using doses and timing that mimic the childhood immunization schedule induces mouse strain-specific effects on weight gain, locomotor and exploratory activity, stereotypic behaviors, and size of CA regions of hippocampus. SJL/J strain mice, a strain with heightened sensitivity to autoimmune disease, show the most prominent behavioral and neuropathologic effects. In this strain, the male gender is associated with a more severe outcome. C57BL/6J mice demonstrate an intermediate phenotype, and BALB/cJ mice have minimal deficits. These findings suggest that brain architecture and function may be altered in genetically susceptible hosts following postnatal thimerosal exposure, and support the utility and relevance of this model as a tool for identifying genetic and maturational factors underlying vulnerability to toxin-induced CNS injury and understanding the pathogenesis of human neurodevelopmental disorders.

Population Epidemiology

The Vaccine Adverse Event Reporting System (VAERS)

The Vaccine Adverse Event Reporting System (VAERS) is an epidemiological database that was established by an Act of the US Congress and has been maintained by the CDC/FDA since 1990. A recent review has indicated that by analyzing VAERS using a technique developed by authors from the National Immunization Program (NIP) of the CDC, the VAERS database has good qualitative and quantitative positive predictive value for identifying various types of vaccine-associated adverse events, potential reporting biases and/or confounders have been found to be minimal following the study of many different types of adverse events following immunization, and results from VAERS have been determined to be consistent with those observed from other databases including the CDC's Vaccine Safety Datalink (VSD).

The technique to analyze the VAERS that was developed by authors from the NIP of the CDC involves calculating the incidence rate of reported adverse events following two vaccines administered to similarly aged populations. In the technique, the net numbers of doses distributed from the Biological Surveillance Summaries of the CDC (this number subtracts doses not distributed or returned during the period) are used to estimate the number of doses administered, allowing for a calculation of the incidence rate of reported adverse events to the VAERS database to be undertaken. Then a relative risk ratio can be determined and statistical tests may be performed to determine if the difference in the incidence rate of reported adverse events is statistically significantly different than expected by chance between the two vaccines. The CDC has employed this

technique to analyze the VAERS in the evaluation of several different types of vaccines. Authors from the NIP of the CDC have concluded that shortcomings such as underreporting, difficulty in determining causal relationship, and lack of precise denominators should apply equally to both vaccines under study, and that in the final comparison between the two vaccines, while not perfectly precise, should still provide accurate relative qualitative and quantitative relationships about shifts in numbers of reported adverse events to the VAERS database.

In our initial examination of the VAERS database concerning thimerosal, we evaluated the VAERS to determine whether there was any association between thimerosal-containing childhood vaccines and neurodevelopmental disorders. In order to conduct our analysis of VAERS, the CDC provided us with their Biologic Surveillance Summaries broken down, yearly by vaccine type and manufacturer. As part of our agreement to gain access to such data, the CDC made us agree that we would not release the identities of the vaccine manufacturers we examined because the CDC claims such information is proprietary between themselves and the vaccine manufacturers.

In our analysis of the VAERS database concerning the effect of thimerosal on the incidence rate of adverse events reported to VAERS, we evaluated thimerosal-containing Diphtheria-Tetanus-acellular-Pertussis (DTaP) vaccines administered from 1992-2000 in comparison to thimerosal-free DTaP vaccines administered from 1997-2000. It should be noted that the thimerosal-free DTaP vaccines examined, were those of vaccine that never contained thimerosal as a preservative in the vaccine, since their introduction. We determined that there was a 6-fold statistically significantly ($p < 0.05$) increased incidence rate of autism reported to VAERS following thimerosal-containing DTaP vaccines in comparison to thimerosal-free DTaP vaccines. We employed a number of control adverse events that we believed should not be biologically plausibly linked with increasing doses of mercury from thimerosal-containing DTaP vaccines, and we found that they tended to be reported similarly following both the thimerosal-containing and thimerosal-free DTaP vaccines under study. We concluded our study by suggesting that an association was found between thimerosal-containing childhood vaccines and neurodevelopmental disorders, including autism.

In our second analysis of the VAERS database, we evaluated dose-response curves for the effects of increasing doses of mercury from thimerosal-containing childhood vaccines, and also evaluated another thimerosal-containing vaccine, namely whole-cell Diphtheria-Tetanus-Pertussis (DTP) vaccine, that was administered to children in a similar childhood schedule as our thimerosal-free DTaP vaccines, so as to see if the effects of thimerosal could be observed with a different thimerosal-containing vaccine other than thimerosal-containing DTaP vaccines. We evaluated thimerosal-containing DTaP and whole-cell DTP vaccines (1992-2000), both in comparison to thimerosal-free DTaP vaccines (1997-2000). The methodology for generating dose-response curves from the VAERS consisted of determining after which vaccine dose number was each type of adverse event associated with in the VAERS. In order to increase the numbers for our analyses, we grouped doses together, so that one grouping was among children receiving 37.5 micrograms of mercury from thimerosal-containing vaccines (Doses 1 and 2, combined), and a second grouping was among children receiving 87.5 micrograms of mercury from thimerosal-containing vaccines (Doses 3 and 4, combined). We used a similar methodology to examine our thimerosal-free DTaP

vaccines, so as to provide a background to compare our children receiving thimerosal-containing vaccines. We found consistent increasing risk dose-response relationships for autism following both of our thimerosal-containing vaccines in our comparison to our thimerosal-free DTaP vaccines. In addition, we observed our control adverse events that were not biologically plausible linked with thimerosal did not follow an increasing risk dose-response relationship with additional doses of mercury from thimerosal-containing vaccines.

In our third study of the VAERS database, we combined our dose-response and overall comparison methodologies to evaluate thimerosal-containing DTaP vaccines in comparison to thimerosal-free DTaP vaccines. In this particular analysis, we also evaluated to see if the years examined in our previous studies may have influenced the observed phenomena in VAERS, so both types of vaccines under study were analyzed for the same years from 1997-2001. We observed similar results to those in our previous studies by finding an increasing risk dose-response curve and overall statistically significantly 2.6-fold increased risk of autism following thimerosal-containing DTaP vaccines in comparison to thimerosal-free DTaP vaccines.

In order to allow for independent investigators to evaluate our methods, we have included with this document the raw VAERS and Biological Surveillance Summary data that we used to evaluate autism and some control adverse events reported to the VAERS database following thimerosal-containing vaccines in comparison to thimerosal-free vaccines.

US Department of Education

In our first evaluation of the US Department of Education data, we evaluated the 2001 US Department of Education report to determine the number of children of various ages that had developed neurodevelopmental disorders including autism and speech disorders. We also evaluated the number of children of various ages that had developed control disorders that were not biologically plausibly linked with thimerosal including orthopedic impairments, visual impairments, and deaf-blindness. We determined the prevalence of each of these conditions in the birth cohorts examined based upon the number of births in each birth cohort as per the CDC's live birth surveillance data. We calculated the average mercury dose from thimerosal administered to each child in the birth cohorts examined by evaluating the total mercury doses from thimerosal-containing vaccines administered to each birth cohort from the yearly Biological Surveillance Summaries of the CDC, and divided this number by the number of births in each birth cohort examined from the CDC's yearly live birth surveillance data. The birth cohort years analyzed were 1984, 1985, and 1990-1994. These birth cohorts were chosen because they were the ones in which all the necessary raw data was available for analysis. The results of our analysis showed that there was a direct increasing dose-response relationship between the prevalence of autism and additional average mercury doses from thimerosal-containing childhood vaccines. In contrast, the control conditions we examined in the US Department of Education showed no correlation with additional average mercury doses from thimerosal-containing vaccines administered to children.

In our second analysis of the US Department of Education data, we once again evaluated the 2001 US Department of Education report using similar methodology and reviewed the same raw data as in our previous analysis, but in our new analysis we established the 1984 birth cohort as a baseline-year. By establishing 1984 as a baseline-

year, we compared all subsequent birth cohorts against our baseline for the relative prevalence of autism and the average mercury dose from thimerosal-containing childhood vaccines. This new method of analysis allowed us to be able to quantitatively evaluate the risk of outcomes in the US Department of Education data as a function of mercury doses from thimerosal-containing childhood vaccines. The results of our analysis showed there was a direct increasing risk dose-response for autism following additional doses of mercury from thimerosal-containing childhood vaccines, and we determined that overall there was a statistically significantly increased risk for autism in comparison to our 1984 baseline measurement. The risk of our control outcomes in the US Department of Education data did not correlate with increasing doses of mercury from thimerosal-containing childhood vaccines. In evaluating the slopes and linear regression coefficients we determined from our analysis of the US Department of Education data, we observed similar trends as those we observed in our analysis of the VAERS database.

In our third analysis of the US Department of Education data, we employed similar methodologies as we previously used. We extended the birth cohorts examined, so as to see if this would effect the relationship between the prevalence of autism in comparison to the average mercury dose children received from thimerosal-containing childhood vaccines (birth cohorts: 1981-1985 and 1990-1996). We quantitatively evaluated the risk of autism in comparison to a baseline birth cohort measurement. In addition, we also evaluated Measles-Mumps-Rubella (MMR) vaccine population coverage estimates to see their potential impact on the population prevalence of autism in comparison to the affects observed from thimerosal-containing childhood vaccines. It was determined that there was a close correlation between mercury doses from thimerosal-containing childhood vaccines and the in the prevalence of autism (birth cohorts: 1981-1985, and 1990-1996) from the late 1980s through the mid-1990s. In contrast, there was a close correlation between the number of primary pediatric measles-containing vaccines administered and the prevalence of autism (birth cohorts: 1982, 1985, and 1991-1996) during the 1980s. In addition, it was found that there were statistically significant odds ratios for the development of autism following increasing doses of mercury from thimerosal-containing vaccines (birth cohorts: 1985 and 1990-1995) in comparison to a baseline measurement (birth cohort: 1984). The contribution of thimerosal from childhood vaccines (> 50% effect) was greater than the potentially small contribution of the MMR vaccine on the population prevalence of autism observed in this study.

Vaccine Safety Datalink (VSD)

The CDC has previously analyzed the Vaccine Safety Datalink (VSD) database to examine the relationship between thimerosal-containing childhood vaccines and childhood neurodevelopmental disorders. The VSD is a large-linked database that includes vaccination, clinic, hospital discharge, and demographic data. The VSD was formed as a partnership between CDC and seven large health maintenance organizations (HMOs), and was initiated in 1991 and covers approximately 2.5% of the US population. In the study, the cumulative vaccine-related mercury exposure was calculated at the end of the first, second, third, and six months of life from automated vaccination records (i.e. to evaluate whether being exposed to higher levels of mercury from thimerosal-containing childhood vaccines was a risk factor for childhood neurodevelopmental disorders. The results of this crude analysis conducted by the CDC showed that there

were statistically significantly increasing adjusted relative risk dose-responses for the effects of additional mercury doses from thimerosal for the following neurodevelopmental disorder outcomes including: any neurodevelopmental disorder, stammering, emotional disturbances, language delay, and speech delay.

We have since, as independent investigators, gone and accessed the VSD database, at the request of the US Congress. In our analyses of the VSD database, we evaluated whether our previous analyses of thimerosal-containing and thimerosal-free DTaP vaccines in the VAERS database represented a false-positive signal, since as the CDC has previously described there may be potential limitations with the VAERS database. In our study of the VSD database, we analyzed data generated from HMO members of the VSD including Group Health Cooperative, Northwest Kaiser Permanente, Kaiser Permanente of Northern California, and Kaiser Colorado. To gain access to the VSD, we were required to submit detailed study protocols for review by the CDC as per the requirements for external researchers from the CDC. Subsequently, we obtained approval for our study protocols from the Institutional Review Boards (IRBs) at Group Health Cooperative, Northwest Kaiser Permanente, Kaiser Permanente of Northern California, and Kaiser Colorado. We accessed the VSD at the National Center for Health Statistics, Research Data Center (Hyattsville, Maryland). We conducted our analysis by analyzing the automated VSD database using a SAS database interface.

In order to assemble our cohorts for analysis, we analyzed the data on immunization status to determine children receiving thimerosal-containing or thimerosal-free DTaP vaccines matched this information to outcomes in the VSD database to assemble our thimerosal-containing and thimerosal-free DTaP vaccine cohorts. The data on immunization tracking systems in the VSD undergoes extensive quality review and show high rates of agreement with data obtained from chart reviews. The overall DTaP cohort population examined in the VSD dataset contained 1,085,320 children.

In order for children to become members of either the thimerosal-containing or thimerosal-free DTaP vaccine cohorts analyzed in our study, we required that child had to receive at least three DTaP vaccines (dose range analyzed: 3-5). The requirement of three doses of DTaP vaccine was instituted, so as to ensure that the children examined in our cohorts were seeking healthcare services from the HMOs analyzed. We determined that there were a total of 69,885 children receiving only thimerosal-free DTaP vaccines, and 85,978 children receiving only thimerosal-containing DTaP vaccines. Outcomes in the VSD database are classified according to the *International Classification of Diseases, 9th Revision* (ICD-9) and we analyzed both inpatient and outpatient diagnoses. We also removed all duplicate diagnoses codes for each child in our cohorts. We identified neurodevelopmental disorder code of autism (299.0) in the VSD.

We calculated the incidence rate of each neurodevelopmental disorders in each of our cohorts. We determined the relative risk by dividing the incidence rate of each condition examined in the cohort that only received thimerosal-containing DTaP vaccine by the incidence rate in the cohort that only received thimerosal-free DTaP vaccine. We determined the attributable risk by subtracting the incidence rate of each condition examined in the cohort that only received thimerosal-containing DTaP vaccine from the incidence rate in the cohort that only received thimerosal-free DTaP vaccine.

In performing the statistical analyses, the premise of equal reactivity between cohorts forms the basis of our null hypothesis. The statistical method involved

constructing 2x2 contingency tables, and we employed likelihood ratio chi-square and fisher's exact (total cases < 5) statistical tests to determine statistical significance. In our statistical tests we posit that the number of conditions and the number of children in the cohort receiving thimerosal-free DTaP are the expected values, and the number of conditions and the number of children in the cohort receiving thimerosal-containing DTaP are the observed values. In this analysis, the statistical package contained in SISA and Fisher's Exact (11, 12) were used, and a double-sided p-value < 0.05 was accepted as statistically significant. In addition, p-values and 95% confidence interval (CI)s (when possible) were determined.

We also as part of our assessment of the impact of thimerosal on autism created dose-response curves. In creating our dose response curves, we selected children specified as receiving four doses of either thimerosal-containing or thimerosal-free DTaP vaccines in various combinations. A requirement of four doses was chosen because sufficient numbers of outcomes were available for a dose-response analysis in this category. We established our cohort that received four doses of thimerosal-free DTaP vaccine as a baseline against which we compared children receiving additional doses of mercury from thimerosal-containing DTaP vaccine. Since, we assume that both cohorts under study were similar populations the relative risk at 0 micrograms of mercury was one. In analyzing subsequent doses of mercury, we combined additional doses of mercury from thimerosal-containing DTaP vaccines so that those receiving 25 and 50 micrograms of mercury from thimerosal-containing DTaP vaccines received an average mercury dose of 39.2 micrograms, and those receiving 75 and 100 micrograms of mercury received an average mercury dose of 87.9 micrograms. We examined the incidence rate of neurodevelopmental disorders determined in each subgroup and compared this incidence to our baseline thimerosal-free DTaP vaccine. Microsoft Excel was used to plot our dose-response curves, determine the slopes, and regression coefficients (y-intercept = 1) for each outcome examined.

We employed several controls in order to determine if confounders or biases were present in the overall DTaP vaccine VSD dataset examined. In order to determine if there were confounders in the dataset examined, we evaluated the mean gestation (weeks), birth weight (grams), APGAR scores at one and five minutes following birth, and maternal age at the time of birth in our compiled dataset in comparison to what was observed in our children diagnosed with autism. We determined the relative risk by dividing the observed value for each confounder examined in the overall DTaP vaccine cohort by the value of the confounder in each specific type of neurodevelopmental disorder condition examined in the present study. Our null hypothesis was that each confounder examined should be similar in the overall DTaP vaccine cohort in comparison to our children diagnosed with autism. We used the t-test in SISA to determine statistical significance. In order to determine if biases were present in the thimerosal-containing and thimerosal-free DTaP vaccine cohorts examined in the VSD database, we employed control ICD-9 diagnoses. The first control was a positive hypersensitivity diagnosis (ICD-9: 995.2). This was a positive control, because we hypothesized that the main difference in vaccine formulations under study was whether they contain thimerosal or not. Therefore, since thimerosal has been acknowledge to cause hypersensitivity reactions in some vaccine recipients, this should result in children in the thimerosal-containing DTaP vaccine cohort having a higher incidence rate in comparison to children in the

thimerosal-free DTaP vaccine cohort. The second control was an overall sampling of diagnoses made in the thimerosal-containing DTaP vaccine cohort in comparison to the thimerosal-free DTaP vaccine cohort. This control was designed to determine whether there were significant differences in the healthcare seeking behaviors of children in the thimerosal-containing and thimerosal-free DTaP vaccine cohorts, because such a difference could account for significant differences in diagnoses within each cohort examined in the VSD database. We also used this second control to create a dose-response curve to determine if there were overall significant healthcare seeking differences depending on the amount of mercury from thimerosal administered to children, and also to evaluate whether our dose-response methodology was appropriate, because in either case additional doses of mercury from thimerosal should not effect the overall healthcare seeking behaviors in both cohorts examined.

We found in our cohort receiving a minimum of three doses of thimerosal-containing DTaP vaccine only in comparison to our cohort receiving a minimum of three doses of thimerosal-free DTaP vaccine only, that there was statistically significantly increased risk for autism (relative risk = 27.6, attributable risk = 3.81 per 10,000 children, $p < 0.0001$). Our positive control hypersensitivity condition was statistically significantly elevated (relative risk = 1.66, attributable risk = 21.3 per 10,000 children, $p < 0.0001$), and our health seeking behavior control was statistically significantly decreased (relative risk = 0.69, $p < 0.0001$), in our thimerosal-containing DTaP vaccine cohort in comparison to our thimerosal-free DTaP vaccine cohort. We showed there was an increasing dose-response curves for autism (exponential regression coefficient = 0.99; slope = $e^{0.025}$) following additional mercury doses from thimerosal-containing DTaP vaccines. The confounders analyzed occurred clinically similarly in the general DTaP cohort in comparison to those children diagnosed with autism. In our control dose-response curve we found that there was no increasing dose-response relationship between additional doses of mercury from thimerosal-containing DTaP vaccines and healthcare seeking behaviors in the cohorts examined (linear regression coefficient = 0.99; slope = -0.0025).

In order to allow for independent investigators to evaluate our methods, we have included with this document raw VSD data that we used in our evaluation of thimerosal-containing DTaP vaccines in comparison to thimerosal-free DTaP vaccines.

California Thimerosal and Autism Ecological Analysis

Blaxill has conducted an ecologic analysis comparing the estimated average cumulative dose of mercury exposure (i.e., the average ethylmercury dose, calculated by multiplying the amount of thimerosal in the various vaccines by the vaccine-specific coverage rate for US children aged 19 to 35 months, by birth year cohort) to the estimated prevalence of autism in children in California per 10,000 population, by birth year. The prevalence of autism was defined as occurrence of persons with autism or other pervasive developmental disorders (PDD), based on an individualized client developmental evaluation performed at intake into the California Department of Developmental Services regional and developmental center system during 1987-1998 and coded as ICD-9 codes 2991, 299.80, or 299.88. The graphical presentation of Blaxill's data showed that the number of children in California coded as having autism-like disorders seeking special education services remained reasonably constant through the mid-1980s, began to rise slightly in 1988, then began to rise more dramatically in 1990, and actually began a slight decrease in the mid-1990s. This graphical pattern directly

correlates with the doses of mercury children received from thimerosal-containing childhood vaccines, and is marked agreement with our own ecological analyses in the US Department of Education, which have shown similar increasing and decreasing trends in the prevalence of autism following changes in the amount of mercury children received from thimerosal-containing childhood vaccines.

Clinical Evaluations of Autistic Children

Mercury Retention Studies

Gale et al. have evaluated the relative activities in mobilizing and promoting excretion of mercury in mercury-laden mice following administration of *meso*-2,3-dimercaptosuccinic acid (DMSA) and 2,3-dimercaptopropane-1-sulfonate (DMPS). In the first evaluation undertaken by the authors, oral treatment was initiated with DMSA and DMPS given for two consecutive days in mice that were loaded with radioactively labeled mercury. The whole-body radioactivity of each mouse was measured 24 hours after each treatment, and it was observed that there were statistically significantly ($p < 0.05$) decreased whole-body mercury levels following DMSA (16% reduction) and DMPS (27% reduction) in comparison to untreated mercury loaded mice. In addition, the cumulative amount of mercury excreted in urine was found to be increased after 3 days of treatment with DMSA (22% increase) and DMPS (85% increase) in comparison to untreated mercury loaded controls. Parallel daily measurements of retained whole-body radioactivity from radiolabeled mercury were in good agreement with the values calculated from the excretion data.

Zhang employed DMPS and DMSA chelation therapy in 27 patients with ethylmercury chloride poisoning. After therapy over a period of 2 months, all had some relief; 19 became asymptomatic, but two cases showed only slight improvement. In addition to the 27 cases treated with chelation, there were 13 cases that were not treated. Two months later, the untreated patients showed little improvement in symptoms and signs. It was observed that during chelation therapy, the urinary mercury levels increased in almost all cases, and the extent of increased urinary mercury was generally consistent with the extent of intoxication ($p < 0.01$). The author concluded that it appears chelation therapy is not only helpful in diagnosis but also aids in assessing the level of intoxication.

Bradstreet et al. examined the concentration of mercury in the urine among children with autistic spectrum disorders in comparison to an age and sex matched normal control children following a three-day treatment with an oral chelating agent, DMSA. It was observed that there was a 3.15-fold statistically significantly ($p < 0.0002$) increased concentration of mercury among 221 children with autistic spectrum disorders in comparison to 18, age and sex matched, normal control children. Among vaccinated children with autistic spectrum disorders, there was a 5.9-fold statistically significantly ($p < 0.005$) elevated urinary mercury concentrations, but similar urinary cadmium and lead concentrations, compared to age and sex matched normal vaccinated controls following DMSA treatment. In addition, it was found that among normal vaccinated controls in comparison to age and sex matched normal unvaccinated controls, there were similar urinary mercury concentrations. In order to allow for independent investigators to evaluate our methods, we have included with this document the raw data that we used to evaluate urinary heavy metal concentrations following chelation in children with autistic spectrum disorders and among normal control children.

A study by Holmes et al. has also evaluated differential rates of postnatal mercury elimination. First baby haircut samples were obtained from 94 children diagnosed with autism and 45 age- and gender-matched controls. Information on diet, dental amalgam fillings, vaccine history, Rho D immunoglobulin administration, and autism symptom severity was collected through maternal survey questionnaire and clinical observation. Hair mercury levels in the autistic group were 0.47 ppm versus 3.63 ppm in controls, a statistically significant difference. The mothers in the autistic group had significantly higher levels of mercury exposure through Rho D immunoglobulin injections and amalgam fillings than control mothers. Within the autistic group, hair mercury levels varied statistically significantly across mildly, moderately, and severely autistic children, with mean group levels of 0.79, 0.46, and 0.21 ppm, respectively. Hair mercury levels among controls were statistically significantly correlated with the number of mothers' amalgam fillings and their fish consumption as well as exposure to mercury through childhood vaccines, correlations that were absent in the autistic group. Hair excretion patterns were significantly reduced relative to controls. These data cast doubt on the efficacy of traditional hair analysis as a measure of total mercury exposure in a subset of the population. The authors concluded that in light of the biological plausibility of mercury's role in neurodevelopmental disorders, the present study provides further insight into one possible mechanism by which early mercury exposures could increase the risk of autism.

It has been hypothesized that these results are likely due to a decreased ability of children, who go onto develop autistic spectrum disorders, to clear mercury from their systems, resulting in the retention of potentially toxic mercury levels. A possible mechanism for the normal excretion of mercury is thought to involve the binding of mercury compounds to sulfhydryl groups. Therefore, impaired sulphation, possibly a pre-existing genetic condition, may contribute to this observed mercury accumulation in some children who go onto develop autism.

Sulphation Studies

James has evaluated sulphation pathways in autistic children. James has overviewed the methionine transsulfuration pathway. In this pathway it is important to note that cysteine is a conditionally essential amino acid that is dependent on adequate methionine status. A decrease in intracellular methionine effectively increases the requirement for cysteine and a decrease in methionine is often associated with an equivalent decrease in cysteine levels. Methionine transsulfuration to cysteine and glutathione occurs primarily in the liver, the predominant organ for methionine metabolism. Cysteine is the rate limiting amino acid for the synthesis of glutathione; thus, low methionine and cysteine levels reduce glutathione synthesis and are associated with decreased intracellular glutathione. Glutathione possesses a strong ability to bind mercury because of the -SH (thiol) group of cysteine within glutathione. Because the intracellular levels of glutathione (1-8mM) greatly exceed extracellular levels (5-7 micromolar), the concentration gradient across the cell membrane strongly favors cellular export over uptake of glutathione. As a consequence, most cells are not able to take up intact glutathione and rely on the uptake of its precursors for the synthesis of glutathione within the cell. Quantitatively, most glutathione synthesis occurs in the liver where it is constantly exported and degraded by extracellular γ -glutamyltranspeptidase into precursor amino acids for transport to and uptake by extrahepatic tissues. In this way,

glutathione synthesis, and indirectly its ability to bind mercury in peripheral tissues, is directly dependent on glutathione synthesis and export by the liver. A decrease in cysteine availability, such as due to genetic polymorphisms, would be expected to negatively affect the ability to bind mercury in vulnerable tissues, such as the brain, that are dependent on hepatic cysteine for glutathione synthesis. A decrease in glutathione synthesis would increase the vulnerability of these tissues to the effects of mercury, and may have relevance to the neurological dysfunction observed in autistic children. James evaluated the baseline levels of methionine transsulfuration metabolism in the plasma from 20 autistic children in comparison to 33 age matched normal control children. The plasma thiol profile observed in the autistic children was severely abnormal. James observed that autistic children had statistically significantly ($p = 0.001$) decreased concentrations of methionine (37% reduction), cysteine (22% reduction), and total glutathione (48% reduction) in comparison to age matched normal control children.

Independently, Bradstreet et al. retrospectively examined consecutive untreated children with previously established autistic spectrum disorders and admitted to the International Child Development Resource Center (ICDRC). Each child was diagnosed with autism (DSM-IV 299.00) or pervasive developmental disorder (DSM-IV 299.80). A total of 286 children were identified that were tested for plasma cysteine concentrations and a total of 90 children were identified that were tested for plasma sulfate concentrations. For each child the first laboratory test performed was the one included in this analysis. The Arizona State University Institutional Review Board approved the retrospective examination of patients. All laboratory specimens for each children examined were sent blinded to the Great Smokies Diagnostic Lab (Asheville, NC) for testing. Plasma cysteine concentrations and plasma sulfate concentrations were measured in milligram/deciliter (mg/dL). Among the children with autistic spectrum disorders examined, the total number of males, females, mean and range of age in years, mean and range for plasma cysteine and plasma sulfate concentrations were determined. The effect of age and sex to determine if they had any effect on plasma cysteine and plasma sulfate concentrations in children with autism spectrum disorders were also examined. As controls the Great Smokies Diagnostic Lab was contacted. They supplied their normal ranges for both plasma cysteine and plasma sulfate concentrations. The Great Smokies Diagnostic Lab meets or exceeds guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS). In addition, they are inspected by the College of American Pathologist (CAP) and Clinical Laboratory Improvement Amendment (CLIA) reviews their ranges on a routine basis. Per CAP and NCCLS guidelines, the Great Smokies Diagnostic Lab employed a population size of 41 for their cysteine reference range and 52 for sulfate. In order to select normal controls, participants were first screened by an exclusion criteria questionnaire created by the staff physician at Great Smokies Diagnostic Lab. The exclusion criterion was reviewed thoroughly, accepted and signed by the remainder of the staff physicians and by the Chief Medical Officer at Great Smokies Diagnostic Lab before it was employed. Upon completion of the sample collection and analysis, the Office of Quality utilized NCCLS recommended guidelines to biostatistically evaluate the data to create reference ranges. A strict outlier removal strategy was employed. The preliminary ranges were evaluated by comparison with patient data to verify accuracy, and the ranges are also evaluated compared to the coefficient of variance, detectable limits and the scientific literature suggested levels. In

comparing plasma cysteine and plasma sulfate concentrations between cases and controls, the null hypothesis was that both populations should have similar concentrations of plasma cysteine and plasma sulfate. The t-test statistic was employed to determine statistical significance. A double-sided p-value < 0.05 was accepted as statistically significant. It was determined that in the overall case population, plasma cysteine concentrations were similar among males ($2.92 \nabla 0.41$) and females ($2.89 \nabla 0.47$) ($p = 0.69$), and plasma sulfate concentrations were also similar among males ($4.70 \nabla 0.65$) and females ($4.97 \nabla 0.81$) ($p = 0.20$). It was observed that there were similar concentrations of plasma sulfate and cysteine, regardless of the age of the children with autistic spectrum disorders, among both males and females. Statistically significantly decreased mean plasma cysteine (17% reduction, $p < 0.0001$) and plasma sulfate (6% reduction, $p < 0.0002$) concentrations were observed among cases in comparison to controls. In order to allow for independent investigators to evaluate our methods, we have included with this document the raw data that we used to evaluate plasma cysteine and plasma sulfate levels among children with autistic spectrum disorders.

Alberti et al. have examined sulphation levels (i.e. a measure of sulphur pathways in the body) among children with autism in comparison to age and sex matched-normal control children. The authors determined that there were approximately 1/3rd statistically significantly ($p < 0.000013$) lower sulphation levels among 60 autistic children in comparison to 20 aged and sex matched-normal control children.

Yorbik et al. have examined glutathione peroxidase activity in 45 autistic children (39 boys and six girls) in comparison to 41 matched-normal controls (35 boys and six girls). It was observed that autistic children had statistically significantly ($p < 0.05$) reduced erythrocyte glutathione peroxidase activity (24%) and plasma glutathione peroxidase activity (31%) in comparison to matched-normal control children.

Genotypic Studies

Westphal et al. report that there is evidence for the involvement of the glutathione (contains sulphydryl groups) system in the metabolism of thimerosal or its decomposition products (organomercury alkyl compounds). The authors conducted a case-control study where homozygous deletions of Glutathione S-transferase T1 (GSTT1) and glutathione S-transferase M1 (GSTM1) were determined by polymerase chain reaction in the populations examined. The authors found that glutathione S-transferase M1 deficiency was significantly more frequent among patients that were sensitive to thimerosal (65.9%, $P = 0.013$) compared with the healthy control group (49.1%). Glutathione S-transferase T1 deficiency in the thimerosal/mercury group (19.8%) was barely elevated versus healthy controls (16.0%). The combined deletion (GSTT1-/GSTM1-) was markedly more frequent among thimerosal sensitive patients than in healthy controls (17.6% vs. 6.5%, $P = 0.0093$), revealing a synergistic effect of these enzyme deficiencies. The authors concluded that since the glutathione-dependent system was repeatedly shown to be involved in the metabolism of thimerosal decomposition products, the observed association may be of functional relevance.

Godfrey et al. have investigated apolipoprotein-E (apo-E) genotyping as an indicator of heavy metal neurotoxicity. Apo-E genotyping determines the inherited parental epsilon 2, 3, or 4 groups, with six homozygous and heterozygous combinations being found (i.e. , 2/2, 2/3, 2/4, 3/3, 3/4, or 4/4). Isomer , 2 has two cysteine amino acids in its structure, , 3 has one cysteine and one arginine, and , 4 has two arginine amino

acids and no cysteine. Cysteine, with its sulphydryl (-SH) bonds, is potentially able to bind to, and remove metals (e.g. mercury and lead) from tissues, whereas arginine, lacking the -SH bonds, would be unable to do this. The authors examined 400 patients with presumptive mercury-related neuro-psychiatric symptoms. The authors compared their symptomatic cohort with 426 normal controls, and found that there was a significantly ($p < 0.001$) greater proportion of symptomatic patients exhibiting the , 4/4 pattern in comparison to normal controls. Moreover, a significantly lower portion of symptomatic patients fell into the , 2/2 and , 2/3 groups ($p < 0.001$) in comparison to normal controls. The authors also chelated 150 symptomatic patients with 2,3-dimercapto-propane sulfonate (DMPS) in comparison to 10 asymptomatic controls, and found that symptomatic patients had 9-times greater urinary mercury concentrations than controls.

Distribution of Thimerosal & Ethylmercury in the Body

Takeda et al. have evaluated mercury compounds in the blood of rats treated with ethylmercuric chloride. The authors administered radiolabeled ethylmercuric chloride to rats, and the chemical nature of the mercury compound that accumulated in the blood was investigated. The authors observed that more than 97% of the total mercury in the blood of the ethylmercuric chloride-treated rats was extracted with dithizone as organic mercury dithizonate and the organic mercury compounds liberated as the chloride from blood was identified by comparison with authentic ethylmercuric chloride. The ethylmercury residue in the blood was found to be bound to hemoglobin, and it was detected as *S*-ethylmercuric cysteine in the pronase digest of the bound hemoglobin. The authors concluded from these results that the ethylmercury residue should accumulate in the blood, binding with sulphydryl groups of cysteine residues by a mercaptide linkage to the hemoglobin molecule. *In vitro* experiments by the authors determined that the distribution of ethylmercuric chloride in the blood was consistent with the high affinity of ethylmercuric chloride for the inside of the membrane of erythrocytes as was seen *in vivo*, and that the mercury compound, once combined with hemoglobin, was transferred with difficulty through the stroma.

Gasset et al. showed administration of thimerosal to animals resulted in a substantial concentration of mercury present in the blood and tissues (including the brain) of treated animals and their offspring, and concluded that thimerosal crosses the blood-brain and placenta barriers. Slikker from the FDA has confirmed that thimerosal crosses the blood-brain and placental barriers and results in appreciable mercury content in tissues including brain.

Blair et al. reported on squirrel monkeys that were dosed intranasally with saline or thimerosal daily for six months. The total amounts of thimerosal given during the six-month period were 418 μ g (low dose group) and 2,280 μ g (high dose group). This was equivalent to 207 and 1,125 μ g mercury. The dose differential was achieved by more frequent administration to the high dose group. Mercury concentrations were significantly raised over control values in brain (high dose group only), liver muscle, and kidney, but not in blood [The authors reported that some control tissue mercury levels were higher than those in the low dose group. Two control monkeys had high brain total mercury levels (169 and 115 ng/g). Without these the group mean value was 19 ng/g compared to 32 ng/g in the low dose group. The authors suggested that the control animals with high mercury levels may have previously been exposed to mercury before

they received them since the animals were of uncertain history, and because mercury can remain in tissues for a longtime]. The authors concluded that accumulation of mercury from chronic thimerosal-preserved medicines is viewed as a potential health hazard for man.

Miller et al. have observed in rats that traces (maximum observed: ~2 ppm) of mercury were present in the brain following injection of 3.0 mg of ethylmercury per kilogram of body weight. It should be noted that this observed value shows that the concentration of mercury in the brain at least approximates the expected brain concentration assuming that body was pure water, and the mercury was allowed to equally distribute to all regions of the body.

Yonaha et al. evaluated the distribution of ethylmercury chloride in mice. The authors reported that ethylmercury chloride was rapidly taken up into the brain of mice. Manifestation of specific neural symptoms was observed from the 9-11th day following administration of ethylmercury chloride (60 ppm – 13 days). The content in the brain at onset of symptoms after administration of ethylmercury chloride was about 21-24 :g mercury / g brain. The authors also observed that in brain of mice administered ethylmercury chloride; a significant part of mercury was accumulated in the organic form.

Molecular Evaluations of the Effects of Mercury on Neuron Degeneration

Marquis isolated gain axons from the circumesophageal connectives of the lobster to determine the effects of the thiol reagent, Mercurochrome, on the excitability of the axonal membrane. Intracellular microelectrode recordings demonstrated that Mercurochrome depolarized the resting membrane potential only slightly, but completely blocked conduction of the propagated action potential. Partial conduction block was markedly potentiated by repeated electrical stimulation. Treatment of the axons with a disulphide reducing agent prior to the application abolished the potentiating effects of stimulation, and also decreased the sensitivity of the nerve fibers to Mercurochrome possibly through the involvement of non-specific disulphide groups.

Baskin et al. demonstrated that micromolar concentrations of thimerosal induced membrane and DNA damage, and initiated caspase-3 dependent apoptosis in human neurons and fibroblasts at concentrations as low as 1 : M, which is equivalent to 201 : g/L of inorganic mercury. Ball et al. in 2001 reported that, in the United States, up to 200 : g of ethylmercury were received in vaccinations by six months of age. The average six-month-old child weighs 5.55 kg, resulting in a dose of 36 : g/kg (200 : g ethylmercury/5.55 kg). Therefore, the concentration studied by Baskin et al. is only about 6-times higher than that received by children from thimerosal-containing immunizations in the first six months of life in the US.

Leong et al. recently examined neurite outgrowth following exposure to the same concentrations of mercury, aluminum, lead, cadmium, and manganese, demonstrating that nanomolar concentrations of mercury markedly disrupted membrane structure and linear growth rates of imaged neurites in 77% of all nerve growth cones. It was observed that the tubulin/microtubule structure disintegrated following nanomolar mercury exposure. In contrast, exposure to other metal ions did not affect growth cone morphology, or their motility rate. The authors reported in the presence of mercury, neuronal somata failed to sprout, whereas the other metals examined did not affect growth patterns of the neurons examined providing visual and biochemical evidence

strongly implicating mercury as an etiological factor in neurodegeneration. Similar results have been observed in tissue culture systems with thimerosal by a number of authors.

In considering the concentration of mercury (36 ng/L) used by Leong et al., assuming the body is composed completely of water and mercury equally distributes throughout the body, as an example, a 2 month child (average weight is 5 Kg) receiving a single DTP vaccine (22 :g of inorganic mercury) received (22 :g/5 Kg) 122-fold in excess of the concentration of mercury used by Leong et al. to cause significant neuron degeneration.

Molecular Evaluations of the Effects of Mercury on Neuron Degeneration with other Substances

Haley and Lovell have evaluated the synergistic toxicities of thimerosal, aluminum hydroxide, neomycin, testosterone, and estrogen in a neuron system. They demonstrated that at 24 hours applications resulted in the following approximate percentages of neurons surviving: 40% (50 nM thimerosal solution), 80% (1.75 ug neomycin/mL solution), 90% (500 nM aluminum hydroxide), and 95% (control). They then combined the applications and demonstrated that at 24 hours applications resulted in the following approximate neurons surviving: 10% (50 nM thimerosal and 500 nM aluminum hydroxide solution), 10% (50 nM thimerosal and 1.75 :g Neomycin/mL), and 0% (50 nM thimerosal, 500 nM aluminum hydroxide, and 1.75 :g Neomycin/mL solution). Haley and Lovell observed that when testosterone was added with thimerosal to their neuron system, it considerably enhanced the toxic effects of thimerosal resulting in 0% neuronal survival within minutes following application of the solution, whereas when estrogen was added with thimerosal in their neuron system, it considerably reduced the toxic effects of thimerosal resulting in greater neuron survival for longer periods following the application of the solution.

Czlonkowska et al. have reviewed the influence of estrogen on neurodegenerative processes. The authors reported that there are gender differences have been associated with decreased risk, delayed onset and progression, or enhanced recovered from numerous traumatic or chronic neurological and mental diseases. It has been determined that locally produced estrogen in the brain has been shown to play a very important role in the development of the central nervous. In the developing brain, estrogens control the differentiation and plasticity of distinct neuronal populations. Estrogens promote growth in the hypothalamus, hippocampus, midbrain and cortex. The neuroprotective effects of estrogens may involve, at least in part, modulation of the expression of molecules involved in the control of apoptosis. In addition to preventing neuronal death, estrogens may also promote axonal growth and neuroregeneration. The influence of estrogens on neuroregenerative may be mediated by the up-regulation of many molecules, including cytoskeletal components, which participate in the process of axonal sprouting and axonal target recognition. It is known that estrogens induce the expression of tau in axons, and this may result in the stabilization of microtubules and promotion of axonal growth.

Molecular Evaluations of the Effects of Mercury on Specific Sites of Neuron Degeneration

Li et al. have reported that in previous studies they determined that prolonged treatment of rats with subtoxic levels of mercury as methylmercury hydroxide (MMH) elicited a two- to three-fold increase in renal glutathione content and a three- to four-fold

increase in the mRNA encoding the catalytically active heavy subunit of γ -glutamylcysteine synthetase (GCS), the rate limiting enzyme in GSH synthesis. The authors, since methylmercury is a patented neurotoxicant, investigated the effect of methylmercury mercury treatment on GSH synthesis and the distribution of GCS mRNA expression in the brain. Male C57B1/6 mice were treated for three consecutive days with MMH (3 mg/kg/day, ip). GSH levels in whole brains were increased by two-fold 24 hours following the first injection and remained elevated two to three times control levels after two subsequent MMH treatments. Concomitantly, whole brain GCS mRNA levels were increased by 2.7-fold 24 hours after the third MMH treatment. Reverse transcription *in situ* PCR amplification of GCS heavy subunit mRNA in brain slices taken from MMH-treated mice showed that GCS expression was selectively localized to the cerebellum and hippocampal regions and, within these regions, to areas which are known to resist methylmercury toxicity. In contrast, no GCS mRNA expression was found in brain regions, which are known to be highly susceptible to mercury toxicity. These findings suggest that resistance to methylmercury toxicity in the brain may reflect the ability of specific neuronal cell types to up-regulate GSH synthesis as a protective response to mercury-mediated cell damage.

Ueha-Ishibashi et al. have compared the effect of thimerosal and methylmercury on their ability to alter glutathione concentrations in cerebellar neurons. The authors observed that increasing micromolar doses of thimerosal and methylmercury similarly decreased the glutathione content of neuron cells in a concentration-dependent manner.

Gustafsson et al. have investigated the occurrence of testosterone receptors in rat brain. They reported that the metabolism and binding of testosterone in male and female rat brain was studied in an attempt to find an explanation for the relative androgen unresponsiveness characterizing the female hypothalamo-pituitary axis involved in regulation of hepatic steroid metabolism. The most significant sex differences in the pattern of testosterone metabolites recovered from several brain regions (including pituitary, pineal gland, and hypothalamus) after intraperitoneal administration of testosterone were the predominance of testosterone and androstenedione in male brain compared to the quantitative importance of androstane, epitestosterone, and dihydroepitestosterone in female brain. One possible explanation for the androgen unresponsiveness of female rats is, therefore, the faster metabolism of testosterone to inactive compounds. It was also shown by experiments that high affinity; low capacity binding sites for testosterone were present in the male pituitary, pineal gland, and hypothalamus. No steroid-binding proteins of similar nature were found in pituitary, pineal gland, or hypothalamus from female rats. The authors concluded that the presence of testosterone receptors in the male brain is the result of neonatal programming ("imprinting") by testicular testosterone.

Metabolic/Perfusion Imaging in Autistic Children

Ryu et al. performed a retrospective review in young children in search of common functional and anatomical abnormalities with brain single-photon emission tomography (SPECT) using technetium-99m ethyl cysteinate dimer (^{99m}Tc -ECD) and correlative magnetic resonance imaging (MRI). The patient population was composed of 23 children aged 28-92 months (mean: 54 months) who met the diagnostic criteria of autism as defined in the DSM-IV and CARS. Brain SPECT was performed after intravenous injection of 185-370 MBq of ^{99m}Tc -ECD using a brain-dedicated annular

crystal gamma camera. MRI was performed in all patients, including T1, T2 axial and T1 sagittal sequences. SPECT data were assessed visually. Twenty patients had abnormal SPECT scans revealing focal areas of decreased perfusion. Decreased perfusion of cerebellar hemisphere (20/23), thalami (19/23), basal ganglia (5/23) and posterior parietal (10/23), and temporal (7/23) areas were noted on brain SPECT. By contrast all patients had normal MRI finding without evidence of abnormalities of the cerebellar vermis, cerebellar hemisphere, thalami, basal ganglia, or parietotemporal cortex. The authors concluded that extensive perfusion impairments involving the cerebellum, thalami, and parietal cortex were found. SPECT may be more sensitive in reflecting the pathophysiology of autism than MRI.

Starkstein et al. examined specific deficits of cerebral perfusion in autistic patients as measured with ^{99m}Tc- ECD SPECT scanning. The study, conducted in an outpatient clinic setting, included a consecutive series of 30 patients with autism and 14 patients with mental retardation but not autism that were comparable in chronological age, mental age, height, weight, and head circumference. All participants were examined with a comprehensive psychiatric and neuropsychological battery and received a ^{99m}Tc- ECD SPECT scan. Autistic patients had significantly lower perfusion than the control group in the following brain regions: right temporal lobe (basal and inferior areas), occipital lobes, thalami, and left basal ganglia. The study demonstrated significant perfusion deficits in specific areas of moderately to severely mentally retarded autistic patients.

These metabolic/perfusion scans of children with autistic spectrum disorders showed damage in similar areas to those areas that have been shown to be damaged by mercury, are those areas in which the brain is afforded minimal protection against the effects of mercury (i.e. they produce minimal glutathione levels), are areas that have been demonstrated to have testosterone receptors resulting in the buildup of significant testosterone concentrations (i.e. testosterone has been shown to potentiate thimerosal neuronal toxicity, whereas estrogen has been shown to reduce thimerosal neuronal toxicity), and the damage observed is consistent with that observed in neuron tissue culture systems following extremely low dose mercury exposure (i.e. neuron functional abnormalities, as apposed to complete structural neuron obliteration).

Politics

The Public Health Service (which includes the FDA, the CDC and the National Institutes of Health) and the American Academy of Pediatrics issued a statement in July 1999 “urging” vaccine makers to reduce or eliminate thimerosal because of “theoretical potential for neurotoxicity.” Last year, the staff for Rep. Dan Burton (R-Ind.) obtained an internal e-mail written June 29, 1999, by former FDA scientist Peter Patriarca. In that e-mail Patriarca offered his colleagues a “pros and cons” assessment of the thimerosal statement shortly before its release: “Will raise questions about FDA being ‘asleep at the switch’ for decades, by allowing a potentially hazardous compound to remain in many childhood vaccines, and not forcing manufacturers to exclude it from new products. Will also raise questions about various advisory bodies about aggressive recommendations for use. We must keep in mind that the dose of ethyl mercury was not generated by ‘rocket science’: conversion of the % of thimerosal to actual ug [micrograms] of mercury involves 9th grade algebra. What took the FDA so long to do the calculations? Why didn’t CDC and the advisory bodies do these calculations while rapidly expanding the childhood immunization schedule?” Roger Bernier, of the CDC’s NIP, received the e-

mail. In a recent interview he explained why the cumulative amount of mercury was never figured. “Vaccines tend to be evaluated on an individual basis, the requirements for safety and efficacy on an individual basis,” Bernier said. “This holistic view of safety was not part of the review.”

The following are excerpts of the transcribed minutes to the Simpsonwood meeting held June 7-8, 2000 in Norcross, Georgia, that were obtained under the Freedom of Information Act, where the finding of the Vaccine Safety Datalink analysis of Thimerosal containing vaccines and neurodevelopmental outcomes were reviewed by a panel of experts in private by the government and industry.

Dr. Verstraeten: Page 31: “It is sort of interesting that when I first came to the CDC as a NIS officer a year ago only, I didn’t really know what I wanted to do, but one of the things I knew I didn’t want to do was studies that had to do with toxicology or environmental health. Because I thought it was too much confounding and it’s very hard to prove anything in those studies. Now it turns out that other people also thought that this study was not the right thing to do, so what I will present to you is the study that nobody thought we should do.”

Dr. Verstraeten: Page 40: “...we have found statistically significant relationships between the exposures and outcomes for these different exposures and outcomes. First, for two months of age, an unspecified developmental delay, which has its own specific ICD-9 code. Exposure at three months of age, Tics. Exposure at six months of age, an attention deficit disorder. Exposure at one, three and six months of age, language and speech delays, which are two separate ICD-9, codes. Exposure at one, three and six months of age, the entire category of neurodevelopmental delays, which includes all of these plus a number of other disorders.”

Dr. Weil: Page 75: “I think that what you are saying is in term of chronic exposure. I think that the alternative scenario is that this is repeated acute exposures, and like many repeated acute exposures, if you consider a dose of 25 micrograms on one day, then you are above threshold. At least we think you are, and then you do that over and over to a series of neurons where the toxic effect may be the same set of neurons or the same set of neurologic processes, it is conceivable that the more mercury you get, the more effect you are going to get.”

Dr. Chen: Page 151: “One of the reasons that led me personally to not be so quick to dismiss the findings was that on his own Tom independently picked three different outcomes that he did not think could be associated with mercury (conjunctivitis, diarrhea and injury) and three out of three had a different pattern across different exposure levels as compared to the ones that again on a priority basis we picked as biologically plausible to be due to mercury exposure.”

Dr. Verstraeten: Page 162: “You are asking for biological plausibility?” Dr. Brent: Page 162: “Well, yes” Dr. Verstraeten: Page 162: “When I saw this, and I went back through the literature, I was actually stunned by what I saw because I thought it is plausible.”

Dr. Johnston: Page 198: “This association leads me to favor a recommendation that infants up to two years old not be immunized with Thimerosal containing vaccines if suitable alternative preparations are available...My gut feeling? It worries me enough. Forgive this personal comment, but I got called out at eight o’clock for an emergency call and my daughter-in-law delivered a son by C-Section. Our first male in the line of the

next generation, and I do not want that grandson to get a Thimerosal containing vaccine until we know better what is going on. It will probably take a long time. In the meantime, and I know there are probably implications for this internationally, but in the meantime I think I want that grandson to only be given Thimerosal-free vaccines.”

Dr. Weil: Page 207: “The number of dose related relationships are linear and statistically significant. You can play with this all you want. They are linear. They are statistically significant. The positive relationships are those that one might expect from the Faroe Islands studies. They are also related to those data we do have on experimental animal data and similar to the neurodevelopmental tox data on other substances, so that I think you can’t accept that this is out of the ordinary. It isn’t out of the ordinary...The increased incidence of neurobehavioral problems in children in the past few decades is probably real...I work in the school system where my effort is entirely in special education and I have to say that the number of kids getting help in special education is growing nationally and state by state at a rate we have not seen before.”

Dr. Brent: Page 229: “The medical legal findings in this study, causal or not, are horrendous and therefore, it is important that the suggested epidemiological, pharmacokinetic, and animal studies be performed. If an allegation was made that a child’s neurobehavioral findings were caused by Thimerosal containing vaccines, you could readily find a junk scientist who would support the claim with “a reasonable degree of certainty”. But you will not find a scientist with any integrity who would say the reverse with the data that is available. And that is true. So we are in a bad position from the standpoint of defending any lawsuits if they were initiated and I am concerned.”

Dr. Clements: Page 247: “I am really concerned that we have taken off like a boat going down one arm of the mangrove swamp at high speed, when in fact there was not enough discussion really early on about which way the boat should go at all. And I really want to risk offending everyone in the room by saying that perhaps this study should not have been done at all, because the outcome of it could have, to some extent, been predicted, and we have all reached this point now where we are left hanging, even though I hear the majority of consultants say to the Board that they are not convinced there is a causality direct link between Thimerosal and various neurological outcomes. I know how we handle it from here is extremely problematic. The ACIP is going to depend on comments from this group in order to move forward into policy, and I have been advised that whatever I say should not move into the policy area because that is not the point of this meeting. But nonetheless, we know from many experiences in history that the pure scientist has done research because of pure science. But that pure science has resulted in splitting the atom or some other process, which is completely beyond the power of the scientists who did the research to control it. And what we have here is people who have, for every best reason in the world, pursued a direction of research. But there is now the point at which the research results have to be handled, and even if this committee decides that there is no association and that information gets out, the work that has been done and through the freedom of information that will be taken by others and will be used in ways beyond the control of this group. And I am very concerned about that as I suspect it is already too late to do anything regardless of any professional body and what they say...”

Dr. Bernier: Page 113: “We have asked you to keep this information confidential...So we are asking people who have done a great job protecting this information up until now, to continue to do that until the time of the ACIP meeting...That

would help all of us to use the machinery that we have in place for considering these data and for arriving at policy recommendations.”

In 2003, a report was published that was prepared by the staff of the Subcommittee on Human Rights and Wellness Committee on Government Reform from the United States House of Representatives on mercury in medicine (This report is the result of a three year investigation initiated in the Committee on Government Reform). Some of the findings of this reported included: One, mercury is hazardous to humans. Its use in medicinal products is undesirable, unnecessary and should be minimized or eliminated entirely. Two, manufacturers of vaccines and thimerosal, (an ethylmercury compound used in vaccines), have never conducted adequate testing on the safety of thimerosal. The FDA has never required manufacturers to conduct adequate safety testing on thimerosal and ethylmercury compounds. Three, studies and papers documenting the hypoallergenicity and toxicity of thimerosal (ethylmercury) have existed for decades. Four, autism in the United States has grown at epidemic proportions during the last decade. By some estimates the number of autistic children in the United States is growing between 10 and 17 percent per year. The medical community has been unable to determine the underlying cause(s) of this explosive growth. At the same time that the incidence of autism was growing, the number of childhood vaccines containing thimerosal was growing, increasing the amount of ethylmercury to which infants were exposed threefold. Five, The FDA and the CDC failed in their duty to be vigilant as new vaccines containing thimerosal were approved and added to the immunization schedule. When the Hepatitis B and Haemophilus Influenzae Type b vaccines were added to the recommended schedule of childhood immunizations, the cumulative amount of ethylmercury to which children were exposed nearly tripled. Six, The amount of ethylmercury to which children were exposed through vaccines prior to the 1999 announcement exceeded two safety thresholds established by the Federal government for a closely related substance – methylmercury. While the Federal Government has established no safety threshold for ethylmercury, experts agree that the methylmercury guidelines are a good substitute. Federal health officials have conceded that the amount of thimerosal in vaccines exceeded the EPA threshold of 0.1 micrograms per kilogram of bodyweight. In fact, the amount of mercury in one dose of DTaP or Hepatitis B vaccines (25 micrograms each) exceeded this threshold many times over. Federal health officials have not conceded that this amount of thimerosal in vaccines exceeded the FDA’s more relaxed threshold of 0.4 micrograms per kilogram of body weight. In most cases, however, it clearly did. Seven, the CDC in general and the National Immunization Program in particular are conflicted in their duties to monitor the safety of vaccines, while also charged with the responsibility of purchasing vaccines for resale as well as promoting increased immunization rates. Eight, to date, studies conducted or funded by the CDC that purportedly dispute any correlation between autism and vaccine injury have been of poor design, under-powered, and fatally flawed. The CDC’s rush to support and promote such research is reflective of a philosophical conflict in looking fairly at emerging theories and clinical data related to adverse reactions from vaccinations.

On 31 October 2003, Congressman Dr. Weldon wrote a letter to the director of the CDC, Dr. Julie Gerberding, about concerns that he had about the four year evolution of the study conducted by Verstraeten et al. from the CDC regarding the relationship between thimerosal and neurodevelopmental disorders. He stated that his review of the

documents concerning this study dating back to February 2000, left him very concerned that rather than seeking to understand whether or not some children were exposed to harmful levels of mercury in childhood vaccines in the 1990s, there may have been selective use of the data to make associations in the earliest evaluations disappear. The following are some very troubling points about the evolution of this study.

First, the lead author of the article, Dr. Thomas Verstraeten, worked for the CDC until he left over two years ago to work in Belgium for GlaxoSmithKline (GSK), a vaccine manufacturer facing liability over thimerosal containing vaccines (TCVs). In violation of their own standards of conduct, *Pediatrics* failed to disclose that Verstraeten is employed by GSK and incorrectly identifies him as an employee of the CDC.

Second, the first version of the study, produced in February 2000, found a significant association between exposure to TCVs and autism and neurological developmental delays (NDDs). It was observed that among children exposed to the higher levels of mercury by three months of age, the study found a relative risk for autism of 2.48. A June 2000 version of the study applied various data manipulations to reduce the autism association to 1.69. In this new version of the study, the authors went outside of the VSD database to secure data from a Massachusetts Health Management Organization, Harvard Pilgrim (HP) in order to counter the association found between TCVs and speech delay. At the that point HP's data was brought in, HP was in receivership by the state of Massachusetts, its computer records had been in shambles for years, it had multiple computer systems that could not communicate with one another, and it used a health care coding system totally different from the one used across the VSD. There are questions relating to a significant underreporting of autism in Massachusetts. In June 2000 a meeting was held in Simpsonwood, GA involving the authors of the study, representatives of the CDC, and the vaccine industry. A review of the transcript of this meeting included some of the following comments. There was a statistically significant relationship was found between exposures and outcomes. There was certainly an under ascertainment of adverse outcomes because some children were just simply not old enough to be diagnosed. There was the possibility of excluding the lowest exposure children from the dataset, and removing those children that got the highest exposure levels since they represented an unusually high percentage of outcomes. The data could be pushed and pulled to get any results. The final published version of the Verstraeten et al. study found a relative risk for autism among the highest exposure group by three months of age of 1.38. The authors concluded that, "No consistent significant associations were found between TCVs and neurodevelopmental outcomes," and that the lack of consistency argues against an association. In reviewing the study, it should be noted that there are data points where children with higher exposures to the neurotoxin mercury actually had statistically significantly fewer developmental disorders (e.g. speech delay at HMO A among children 7 months of age). This demonstrates to me how excessive manipulation of data can lead to absurd results.

Congressman Dr. Weldon concluded his letter by stating that he found a disturbing pattern which merits a thorough, open, timely, and independent review by researchers outside of the CDC, Health and Human Services, the vaccine industry, and others with a conflict of interest in vaccine related issues (including many in university settings who many have conflicts). Congressman Dr. Weldon stated that he is a strong supporter of childhood vaccinations and know that they have saved us from considerable

death and suffering. A key part of our vaccination program is to ensure that we do everything possible to ensure that these vaccines, which are mandatory, are as safe as possible. We must fully disclose adverse events. Anything less than this undermines public confidence.

Articles Recommending the Removal of Thimerosal from Vaccines and the Continued Presence of Thimerosal in Vaccines

Kravchenko et al. have reported, "Thus thimerosal, commonly used as a preservative, has been found not only to render its primary toxic effect, but also capable of changing the properties of cells. This fact suggests that the use of thimerosal for the preservation of medical biological preparations, especially those intended for children, is inadmissible." Cox and Forsyth stated, "However, individual cases of severe reactions to thimerosal demonstrate a need for vaccines with an alternative preservative." Forstrom et al. indicated, "...reactions can be expected in such a high percentage of merthiolate-sensitive persons that merthiolate in vaccines should be replaced by another antibacterial agent" Seal et al. have published in the *Lancet*, "Thimerosal is a weak antibacterial agent that is rapidly broken down to products, including ethylmercury residues, which are neurotoxic. Its role as a preservative in vaccines has been questioned, and the pharmaceutical industry itself considers its use as historical." Heyworth and Truelove concluded, "For many years, merthiolate has been known to have anti-microbial activity. When it was first introduced as an anti-microbial preservative, little information about the fundamental biological effects of organic mercury compounds was available. We would like to suggest that merthiolate should now be regarded as an inappropriate preservative for anti-lymphocytic globulin preparations and other materials which are indented for administration to human subjects." Winship reported, "Multidose vaccines and allergy-testing extracts contain a mercurial preservative, 0.01% thiomersal, and may occasionally present problems in practice. It is, therefore, now accepted that multidose injection preparations are undesirable and that preservatives should not be present in unit dose preparations." Sanders et al. concluded, "We also recommend that safer alternatives to thimerosal (a mercury sodium salt, 50% mercury) be used to preserve all vaccines."

It is clear that despite some public perception that thimerosal has been removed from all vaccines, it is clearly obvious that thimerosal continues to be present in a number of vaccines at non-trace concentrations. Additionally, the removal of thimerosal from the routinely recommended childhood immunization schedule took considerably longer than is commonly acknowledged.

On July 17, 2003 the Associate Commissioner for Legislation for the FDA wrote a response letter to a March 12, 2003 letter written by Congressman Weldon enquiring about the presence of thimerosal in vaccines. The letter states that the routinely recommended pediatric vaccines (namely, those recommended by the Advisory Committee on Immunization Practices) that are administered during the first 2 years of life (hepatitis B vaccine, inactivated poliovirus vaccine, the 7-valent pneumococcal conjugate vaccine, the Hib vaccine, DTaP, the MMR and varicella vaccine) have only all been thimerosal-free or contain only trace amounts of mercury (< 1 microgram per dose) from thimerosal as a residual from the manufacturing process, since the end of 2002. The letter reviews that there were lots of the following vaccines containing thimerosal throughout 2002 including: Tripedia (DTaP, Aventis Pasteur, 25 micrograms of mercury per dose), Recombivax HB (hepatitis B, Merck, 12.5 or 25 micrograms of mercury per

dose), and Energix B (hepatitis B, 12.5 or 25 micrograms of mercury per dose). The letter also reviews that the following thimerosal-containing vaccines were available in 2003 including thimerosal-containing DT vaccine (Aventis Pasteur, 25 micrograms per dose), thimerosal-containing Td vaccine (for children 7 years of age and older, 25 micrograms of mercury per dose), and thimerosal-containing influenza vaccine (25 micrograms of mercury per dose, Aventis Pasteur and Evans). Additionally, the letter states, "You have asked if it is possible for a child in the U.S. today to get an exposure to 75 micrograms, 100 micrograms, or more of mercury in routine 2, 4, and 6 month well-baby visits. We believe that this is very unlikely." It is clear with the language used by the FDA that some children might still be exposed to significant levels of mercury during the first six months of life from thimerosal-containing vaccines (i.e. As illustrative of possibility that some children may be exposed to significant levels of mercury during the first six months of life from thimerosal-containing vaccines, the FDA states regarding the possibility that children born today could receive 300 micrograms of mercury, "Based on the information available to us at this time, we do not believe a child born today could received 300 micrograms of mercury during the first six years of life the childhood vaccines, including possible influenza vaccines each year").

We have independently purchased vaccines to see, which if any, still contain non-trace amounts of thimerosal. We have found that the following vaccines still contain non-trace amounts of mercury including Meningococcal Polysaccharide Vaccine [Aventis Pasteur, 10 Dose Vial (25 Micrograms of Mercury per Dose), Lot UB505AA - Expires 17 Jun 05], Td Vaccine [Aventis Pasteur, 10 Dose Vial (25 Micrograms of Mercury per Dose), Lot U1014AA - Expires 2 Sept 05], Tetanus Toxoid Absorbed Vaccine Aventis Pasteur, 10 Dose Vial (25 Micrograms of Mercury per Dose), Lot U1048BA - Expires 8 Sept 05], Tetanus Toxoid Vaccine [Aventis Pasteur, 15 Dose Vial, (25 Micrograms of Mercury per Dose), Lot U0775AA - Expires 19 Mar 05], Japanese Encephalitis Virus Vaccine [JE-VAX, Aventis Pasteur, 3 x 1 mL Vial (35.7 Micrograms Mercury per Dose), Lot EJM*196B - Expires 15 Feb 2004], Influenza Virus Vaccine [Fluzone, Aventis Pasteur, 5 mL Vial (25 Micrograms Mercury per Dose), Lot U1130AA - Expires 30 June 2003], Td [Massachusetts Public Health Biological Laboratories, 7.5 mL Vial (8.3 Micrograms of Mercury per Dose), Lot 102 - Expires 21 May 2005], and Pediatric DT Vaccine [Aventis Pasteur, 5 mL Vial (25 Micrograms of Mercury per Dose), Lot U0745AG - Expires 19 February 2004]. Perhaps, one of the most troubling aspects concerning these vaccines listed previously, is that some of them have expiration dates at the end of 2005 (i.e. this means that the vaccine manufacturers still continue to produce thimerosal-containing vaccines, and they will be available to consumers for a long-time into the future).

Conclusion

The evidence presented in this review represents a mere fraction of the many thousands of peer-reviewed articles that have been published by authors from many fields of science and medicine over many decades warning of the dangers of thimerosal. It is clear that the vast evidence in the peer-reviewed literature points to the conclusion that thimerosal has a causal relationship with autism. Regardless of the findings of the Institute of Medicine regarding the relationship between thimerosal and autism, it is clear that thimerosal has no place in medicine. It is also clear, despite claims to the contrary from the American Academy of Pediatrics, the US Public Health Service, FDA, and the

Institute of Medicine, that thimerosal still is present in a number of vaccines at significant concentrations. Thimerosal should be banned for use in humans at any concentrations.

In conclusion, for those who have a decreased ability to excrete mercury, as has been demonstrated for several different genotypes, there can be little doubt that mercury concentrations once administered to children as part of the childhood routine vaccination schedule resulted in a significant number of children developing autism. This is especially true following a sudden increase in the amount of mercury administered, as occurred in the United States in the early 1990s when the amount of mercury administered to children in the first six months of life more than doubled as part of the routine childhood immunization schedule (i.e. from 75 micrograms of mercury from three DTP immunizations to 187.5 micrograms from three DTP, three Hib, and three hepatitis B immunizations). It should be kept in mind that the gene pool contains many susceptible individuals who would have been normal but for the mercury in their vaccines.

It is also clear that if somehow, despite the over whelming evidence, the IOM determines, that either thimerosal did not cause or that they are not sure that it caused the current epidemic of autism and other neurological disorders, that the IOM must demand the immediate expenditure of billions of dollars as part of an all out effort to immediately determine what is causing this epidemic before it totally destroys our society.